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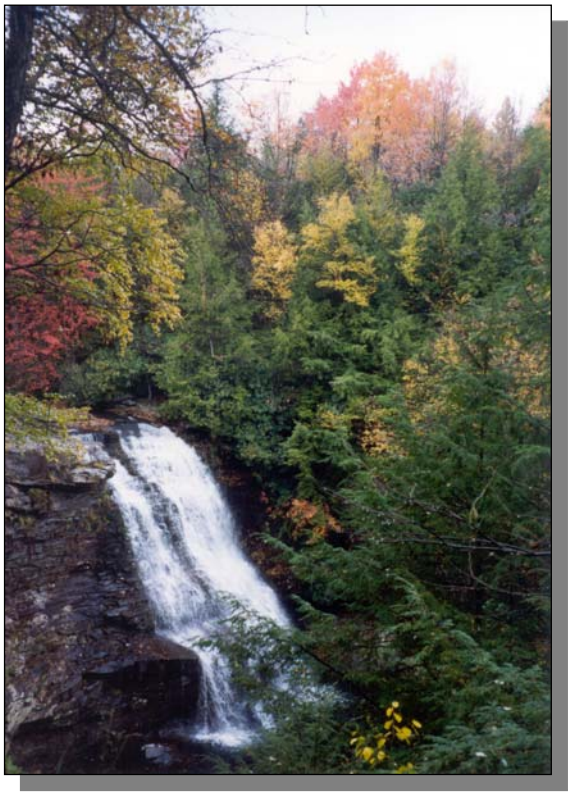

The Hemlock Woolly Adelgid (HWA) newsletter is provided by the USDA Forest Service Northeastern Area State and Private Forestry in support of the HWA Working Group. This informal newsletter provides brief updates for those interested in HWA activities. We encourage readers to contact authors for more detailed information.

Brad Onken (304) 285-1546
USDA Forest Service
bonken@fs.fed.us

Dennis Souto (603) 868-7717
USDA Forest Service
dsouto@fs.fed.us

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THE HWA INITIATIVE IN 2006

Our focus is to develop and implement management tools that minimize the impact of HWA in the Eastern United States. More than $5.7 million was provided by USDA Forest Service State and Private Forestry ($3.3 million) and Research and Development ($2.4 million) to support this effort in 2006. The activities described in this newsletter highlight many of the ongoing efforts. Nearly 75 percent of the funding is directed toward developing and implementing biological and chemical control strategies. The remainder of the funding supports work involving survey and monitoring, host resistance, biology and ecology, and information transfer. Here's a brief overview of biological and chemical control activities:

Biological Control: The primary focus areas this year include 1) exploration in China, Japan, and the Western United States to find new natural enemies; 2) learning how to rear predators on artificial diets; 3) accurately and quickly screening newly discovered predators to ensure their desirability and safety; and 4) releasing and establishing HWA predators and evaluating their impact on HWA and hemlock health. This year, more than 260,000 *Sasajiscymnus tsugae* beetles were reared and released (mostly in the South), and more than 5,000 each of *Laricobius nigrinus* and *Scymnus sinuanodulus* beetles were released at sites from Connecticut to Georgia.

Are the predators being released working? The impact of these predators is extremely difficult to measure and may take decades before we really know. Our goal is to establish a complex of natural enemies that will self perpetuate and prevent HWA outbreaks. Each predator is unique in its dispersal, reproductive potential, feeding behavior, and suitable climate regimes. But they all share two important traits—they are very host specific and have a voracious appetite for HWA.

S. tsugae. Mass rearing and release in multiple States began in 1999 but the earliest releases began in Connecticut in 1995. Reports of recovery in years following release have been sporadic, even though more than 1.5 million beetles have been released in 15 States. Adult beetles have been captured near some release sites more than 6 years after release and in some cases more than ½ mile from the nearest release site. However, the numbers of beetles captured have rarely been more than one or two beetles per site. So far, control of HWA in forest settings has not been clearly demonstrated. See articles by Carole Cheah, Cora Allard, and Jerome Grant in this newsletter for further information.

S. sinuanodulus. Mass rearing and release of this Chinese beetle in multiple States began in 2005, and so far more than 10,000 adult beetles have been released in six States. No beetles have been recovered from release sites yet. See articles by Mike Montgomery and Jennifer DeSio in this newsletter for further information.

L. nigrinus. Mass rearing began in 2003, and more than 15,000 beetles have been released in eight States from Massachusetts to Georgia. Establishment has been confirmed at most sites. At some sites, adult beetles are easily found and hundreds of larvae recovered. See articles by Dave Mausel and Scott Salom in this newsletter for further information.

Chemical Control: Chemical treatments are effective but currently limited to individual tree treatments, making it impractical to treat large hemlock forests. Large hemlock stands will require either effective biological control measures or an acceptable insecticide and application technology that can be effectively applied on a broad scale. In 2006, more than $1.5 million were provided to State and Federal resource managers to protect thousands of "high-value" hemlock trees in recreational and scenic areas, specimen trees, and critical habitat. Imidacloprid has been the chemical of choice in most cases. It is applied as either a soil treatment or injected directly into the tree. Soil treatments have proven to be effective for at least 3 years. Tree injections have been used when hemlocks occur close to water or in areas where soil conditions are not suitable for soil treatments.

Brad Onken (304) 285-1546
USDA Forest Service
bonken@fs.fed.us

BIOLOGICAL CONTROL

Special requests for proposals

In 2005 and 2006, the Northeastern Research Station, Northeastern Area State and Private Forestry, and Region 8 jointly issued calls for research and technology proposals on "Host Plant Resistance to HWA" (2005) and "Advancement of Biological Control of HWA" (2006). In 2005, 19 proposals were received and 7 were funded, totaling $375,000. In 2006, 12 proposals were received and 6 were funded, totaling more than $400,000.

Proposals Funded - 2005	**Principal Investigators/Affiliation**
Evaluating hemlock species for arthropod resistance, hardiness, and cultural adaptability for controlled breeding programs	Ricky M. Bates, James C. Sellmer, Gregory A. Hoover, Pennsylvania State University
Hemlock propagation and evaluation: Vegetative propagation of selected species accessions and interspecific hybrids to permit replicated evaluation of genotype tolerance to hemlock woolly adelgid under different environmental conditions	John Hammond, Susan E. Bentz, USDA ARS U.S. National Arboretum
Single cell EST sequencing to define resistant and susceptible molecular host responses to hemlock woolly adelgid	C. Dana Nelson, Gary F. Peter, Alison M. Morse, John M. Davis, USDA FS SRS, Southern Institute of Forest Genetics
Measurement of phytochemicals in *Tsuga* cultivars and an evaluation of their role in host resistance to the hemlock woolly adelgid (*Adelges tsugae*)	Anthony F. Lagalante, Villanova University

Dendrochronological analyses for determination of host plant resistance to hemlock woolly adelgid (HWA)	James Rentch, West Virginia University
Resistance to hemlock woolly adelgid among local and nonindigenous *Tsuga* populations	Paul A. Weston, Cornell University

Proposals Funded - 2006	**Principal Investigators/Affiliation**
Enhancing biological control of hemlock woolly adelgid using DNA barcodes to identify natural enemies and determine their prey preferences in the field	Adalgisa Caccone, Nathan Havill, Yale University
Evaluation of predators of hemlock woolly adelgid with whole-tree enclosures	Joseph Elkinton, Roy VanDriesche, University of Massachusetts
Large field cage assessments of introduced biological control agents: Survival, interactions, and establishment on hemlock woolly adelgid	Jerome Grant, Paris Lambdin, University of Tennessee
Chamaemyiid predators of the hemlock woolly adelgid in the Pacific Northwest	Darrell W. Ross, Kimberly F. Wallin, Oregon State University
Qualitative and quantitative assessment of introduced biological control agents of hemlock woolly adelgid	Jerome Grant, Paris Lambdin, University of Tennessee
Post-release evaluation of *Laricobius nigrinus* impact on hemlock woolly adelgid	Scott M. Salom, Loke T. Kok, David L. Mausel, Virginia Tech

Kathleen Shields (203) 230-4320
USDA Forest Service
kshields@fs.fed.us

Rearing the Chinese HWA predator *Scymnus sinuanodulus* at the NJDA

The New Jersey Department of Agriculture began rearing *Scymnus sinuanodulus* (*Ss*) in December 2003 with an initial stock of 50 individuals from Carole Cheah's colony at Hamden. From that stock, we've produced nearly 18,000 to date. In 2004, we built a colony to provide enough females for mass production. In 2005 and 2006, we produced 7,000 and 10,000 beetles, respectively, for releases in New Jersey, Pennsylvania, North Carolina, West Virginia, and Maryland.

Because of its lower fecundity and univoltine nature, *Ss* has been more difficult to mass produce than *Sasajiscymnus tsugae*. Females produce fewer eggs and developmental times are longer. In our early experience, *Ss* females were reproductive for only 6 to 8 weeks, after which oviposition stopped for the year. After Carole Cheah advised us that *Ss* oviposits best at the unusually low temperature of 12 °C, the oviposition period was extended to the full term of HWA's active state.

Ss production was increased by the improved efficiency of operation that often arises in developing a rearing procedure, improved understanding of *Ss* rearing requirements, and a stable supply of good quality adelgid. In addition, the host material that we received in a barter agreement with Pennsylvania and North Carolina was consistently viable and plentiful, with regular collections arriving weekly. In previous years, the HWA had experienced high winter mortality, but because of a mild winter, the populations of HWA survived and we were able to depend on a constant and healthy food supply.

Jennifer DeSio (609) 530-4192
New Jersey Department of Agriculture
jennifer.desio@ag.state.nj.us

Hemlock woolly adelgid in Connecticut 2005-2006

Assessments of 2006 winter mortality of HWA were conducted at 15 sites in Connecticut, characterized as a warm winter overall but with an early cold snap which occurred in mid-December. Adelgid mortality was lower than in previous years, averaging 61.4 percent but with great variability from north to south. Hemlock health assessments for 2006 are currently being conducted in *Sasajiscymnus tsugae* release sites in Connecticut. With 50 percent of established sites surveyed, adelgid populations have remained very low or nonexistent (less than 10 percent on whole crowns) in spite of the warmer winter, with hemlock recovery, abundant new growth production, and excellent foliage transparency levels in release sites ranging from northern to coastal sites. In 2005, HWA winter mortality averaged 71 percent for Connecticut. In 2005, recovery and refoliation of hemlocks was also reported statewide but to a lesser degree in previously adelgid-damaged nonrelease stands. Hemlocks in release sites had significantly better crowns than in nonrelease stands.

About 174,000 adult *S. tsugae* have been released at 26 sites in Connecticut from 1995-2006, with four new sites added in 2006 using small releases of *S. tsugae* reared at the Valley Laboratory in Windsor, CT.

Ongoing experiments in collaboration with Dr. Allen Cohen of the Insect and Diet Rearing Institute, Tucson, AZ, are focused on developing an artificial diet presentation system to improve and enhance rearing of *S. tsugae*. Survival of adults on an artificial diet developed by Dr. Cohen has been recorded at 13 weeks, and females were immediately reproductive upon transfer to HWA. Surviving males also mated normally, indicating the nutritional completeness of this formulation.

Carole Cheah (860) 683-4980
Connecticut Agricultural Experiment Station
Carole.Cheah@po.state.ct.us

Evaluation of egg releases to establish *Sasajiscymnus tsugae*

Sasajiscymnus tsugae, imported from Asia for biological control of hemlock woolly adelgid, is currently reared in several facilities. However, the rearing process is costly, time consuming, labor intensive, and is unable to meet the demand. Releasing *S. tsugae* as eggs, rather than adult beetles, has been suggested as a way to reduce labor and costs associated with rearing and to increase production. The germinal idea for egg releases originated at the New Jersey Department of Agriculture. Field and laboratory research was conducted to assess the impact of release timing, egg densities, and predation on the efficiency of egg releases. As expected, cold temperatures greatly reduced survival of *S. tsugae* eggs placed in the field. Timing egg releases with adequate temperature and suitable prey stages is critical to successful survival and colonization of *S. tsugae*. Results suggest that the optimum time for egg release in our region is April. Releases made earlier or later may not have suitable temperature or food for survival and development, as adequate temperature and food must be present at the time of release, as well as several weeks after release. Results support the use of egg releases of *S. tsugae* for field establishment against hemlock woolly adelgid. Eggs hatched, developed through all life stages, and adults were recovered. Predation could adversely affect colonization and establishment of *S. tsugae* via egg releases. Further research will provide a better understanding of release protocol to enable forest resource managers to enhance establishment of *S. tsugae* against hemlock woolly adelgid on eastern hemlock. An egg release protocol (available upon request) was developed to enhance the establishment of predatory beetles via egg releases (in cooperation with the New Jersey Department of Agriculture and USDA Forest Service).

Jerome Grant (865) 974-0218
University of Tennessee
jgrant@utk.edu

Field cage trials of *Scymnus sinuanodulus*

Scymnus sinuanodulus, the lady beetle imported from China, was evaluated in North Carolina on naturally infested hemlock to determine a release protocol and impact on HWA populations. The studies were done in cooperation with the University of Georgia (2005) and Symbiont®, a biological pest management company (2006). The lady beetles were overwintered at 10-12°C in the laboratory and had begun to lay eggs on HWA ovisacs in late winter. This protocol provides for obtaining beetle eggs in the laboratory for continuation of the colony while holding the beetles to the optimal time and condition for deployment in the field (= when average daily temperature is 7-10°C, the temperature initial egg laying was observed in China). The ovisacs of the sistens generation of HWA were counted on the branch, which would be covered by sleeve cages, either without (control) or with lady beetles (3♀ and 2♂ in 2005 and 2♀ and 1♂ in 2006). The cages were removed about 12 weeks later when the progrediens generation was beginning to lay eggs. The numerical change in the adelgid population from one generation to the next in cages with lady beetles was 10-fold lower and 5-fold lower than the controls in 2005 and 2006, respectively. At the end of the test, lady beetle progeny in the bags ranged from 2nd instar larvae to new adults. A degree-day model can be used to determine when to release the lady beetles to predict their development in the field.

Mike Montgomery (203) 230-4331
USDA Forest Service
memontgomery@fs.fed.us

Temporal and spatial activity patterns among three exotic predators of HWA

Robbie Flowers used video studies to document temporal and spatial activity patterns among two specialist predators, *Laricobius nigrinus* (Coleoptera: Derodontidae) and *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae), and a generalist predator, *Harmonia axyridis* (Coleoptera: Coccinellidae), of HWA and how these patterns are affected by additional conspecific and heterospecific predators. Single- and paired-predator assays were tested in the laboratory under simulated spring and summer conditions. Digital video recordings were captured every 15 min over 24 h and scored for the predominant behavior exhibited and relative location of each predator. All species exhibited continuous activity patterns punctuated by longer periods of rest. Predator activity was not coordinated with any particular time of day or location. In spring conditions, *L. nigrinus* had greater activity and a more even behavior distribution than *S. tsugae* or *H. axyridis*, which were skewed towards resting. During the summer, a more even behavioral pattern was seen for the latter two species. Paired-predator assays suggested that conspecifics exert negative influence on one another, leading to increased dispersal movements and decreased resting, feeding, and oviposition. In contrast, heterospecific combinations did not significantly affect predator behavior patterns. A high degree of spatial separation, relative to assay size, was maintained in all conspecific and heterospecific combinations, suggesting that avoidance

behaviors may occur in these species in response to chemical or tactile cues. Overall, these studies suggest that these species are compatible, as particular temporal and spatial patterns were not highly coordinated, and avoidance behaviors were such that significant interspecific interference may be unlikely to occur under more natural conditions. Management implications include applying low-density releases to avoid the potential negative impacts of intraspecific competition.

Scott Salom (540) 231-2794
Virginia Tech
salom@vt.edu

Overseas exploration for biological controls

Several new potential biological controls for HWA were discovered during visits to Japan and China in 2005 and 2006. A new species of *Laricobius* beetle found in high numbers in March and April in Japan has been imported by Scott Salom's group at Virginia Tech for further evaluation in quarantine. Later in the season in Japan, cantharid beetles and syrphid flies were found that appear to have a critical role in driving high-density HWA to low levels; however, they may not be sufficiently host specific for importation as biological controls. Compared to Japan, China has a much greater diversity of predators. Several new species of lady beetles were found in China, and three of these have been imported for further evaluation in the USDA Forest Service Quarantine Laboratory at Ansonia, CT. A predatory hemipteran, *Tetraphleps galachanoides*, that was very abundant on localized, high-density populations of HWA in late spring and summer was shipped to Virginia Tech for colony establishment and further evaluation. Additionally, *Leucopis* sp., syrphids, and several other species of predatory hemipterans have been found in China. Studies of predators in Asia are continuing through cooperative agreements with the Osaka Museum of Natural History, Sichuan Academy of Forestry, Research Institute of Resource Insects in Yunnan, and Beijing Academy of Agricultural and Forest Sciences.

Mike Montgomery (203) 230-4331
USDA Forest Service
memontgomery@fs.fed.us

Establishment of *Laricobius nigrinus* (Coleoptera: Derodontidae) for classical biological control of HWA

A mixed release approach was used to introduce the Pacific Northwest native *Laricobius nigrinus* Fender, a predator of the exotic hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), on eastern hemlock trees from Massachusetts to Georgia. The manipulated factors included (1) the number of beetles released, (2) time of release, and (3) location. There were 10 releases in 2003-2004 consisting of the following replicated treatments:

300 adults in fall 2003, 300 in spring 2004, or 300 in fall 2003 plus spring 2004 (sum = 600). There were 12 releases in 2004-2005, including new release sizes of 75, 150, or 1,200 adults in fall or spring. In fall 2004, beatsheet sampling recovered 4 *L. nigrinus* F_1 adults at 2 of 10 sites. In spring 2005, branch clipping recovered 285 F_2 *Laricobius* larvae from 8 of 10 sites. In fall 2005, beatsheet sampling recovered 13 F_2 and 33 F_1 *L. nigrinus* adults at the 2003-2004 and 2004-2005 sites, respectively (4 of 22 sites). In spring 2006, 2,306 F_2 and F_3 larvae have been collected from 19 of 22 sites. Recoveries of F_3 *L. nigrinus* and increasing recoveries at most sites indicate that this important predator has established permanent populations in the Eastern U.S. Recoveries have been made at all release sites at times but no recoveries have been made in Georgia (the warmest release location), Massachusetts, and one of the western Maryland sites (the coldest release locations). More time is required to monitor long-term population establishment, changing tree health conditions, and HWA populations at these sites to determine an optimal *L. nigrinus* release strategy.

D.L. Mausel (540) 818-5178
Virginia Tech
dmausel@vt.edu

Life cycle synchrony among the predators *Laricobius nigrinus* and *Laricobius rubidus* (Coleoptera: Derodontidae) and their prey, the HWA, in southwestern Virginia

The life cycles of the Pacific Northwest native *Laricobius nigrinus* Fender, Eastern North American native *Laricobius rubidus* LeConte, and HWA were documented in southwestern Virginia to determine temporal synchrony. A 0.4-ha plantation of eastern hemlock, *Tsuga canadensis*, was established in 2001 adjacent to a 10-year-old white pine, *Pinus strobus*, plantation, and was artificially infested with HWA in 2002. In November 2003, 252 adult *L. nigrinus* were released at a rate of 1 to 6 beetles per HWA-infested tree. From September 2005 to July 2006, adult *L. nigrinus* (F_2 generation) and *L. rubidus* were collected with beat sheets at 2-week intervals and their abundance recorded by species. In addition, hemlock foliage clippings infested with HWA were collected from the trees at 1- or 2-week intervals (late February to May). The foliage samples were examined microscopically and the numbers of HWA and *Laricobius* spp. immature stages (F_3 for *L. nigrinus*) were recorded. Beat sheet sampling collected 193 *L. nigrinus* adults from November 3 (2005) to April 10 (2006) and 383 *L. rubidus* adults from November 3 (2005) to May 8 (2006). The appearances of *Laricobius* spp. adults coincided with the resumption of HWA sistens generation development after aestival diapause, and were present through sistens oviposition of progrediens eggs. *Laricobius* spp. eggs and

larvae (sum = 329) were collected from February 24 to May 8 (2006), which matched the onset and end of sistens oviposition. *Laricobius nigrinus* and *L. rubidus* adults and immature stages were highly synchronized with acceptable HWA prey stages. The adaptability of *L. nigrinus* to a new environment and *L. rubidus* to a new host suggests a high potential for successful biological control of HWA.

D.L. Mausel (540) 818-5178
Virginia Tech
dmausel@vt.edu

The Japanese *Laricobius*: Recently discovered and imported to the U.S. as a potential biological control agent for HWA

Several predators are currently being investigated for their potential as biological control agents. Ongoing foreign exploration has revealed several new predators from China, including two *Laricobius* species and several coccinellids. Mike Montgomery (USDA Forest Service) recently initiated collaboration with Shigehiko Shiyake, a Japanese scientist at the Osaka Natural History Museum. While sampling, *Tsuga seiboldii,* a previously unknown *Laricobius* species, was discovered in May 2005. In March 2006, a collection trip was made and over 300 adult beetles were collected and brought back to the U.S. for study. Our current investigation is focused on female oviposition rate, host suitability tests, and developmental studies. Next year, we hope to conduct choice and no-choice feeding and oviposition tests, and more host suitability tests and developmental studies. Some preliminary results include the following: (1) The Japanese *Laricobius* is similar to *L. nigrinus* with respect to life history and behavior; however, there are some differences; (2) *Laricobius* sp. n. exhibited sexual dimorphism; (3) *Laricobius* sp. n. has a higher oviposition rate than *L. nigrinus*; (4) The development of *Laricobius* sp. n. eggs was similar to that of *L. nigrinus*; however, larval development was much faster at 9° and 12 °C than for *L. nigrinus*; (5) In no-choice studies, *Laricobius* sp. n. was able to develop to the 4th instar on BWA and PBA; however, larvae that developed on HWA had a higher survival rate; and (6) We expect to have a viable *Laricobius* n. sp. colony in 2007 since over 5,000 larvae have been reared in the U.S. Rearing procedures will be based on those developed for *L. nigrinus*.

A.B. Lamb (540) 239-0893
Virginia Tech
aslamb@vt.edu

Clemson University: Beetle mass-rearing efforts and HWA research updates

The Clemson University Insectary has mass reared *Sasajiscymnus tsugae* for 3 years. The 2003-04 *S. tsugae* rearing season produced 105,578+ beetles between November 6, 2003, and July 14, 2004. Beetles were released at a total of 41 sites across North Carolina (47,860), South Carolina (36,193), and Georgia (21,525). The 2004-05 *S. tsugae* rearing season produced 211,228+ beetles between November 30, 2004, and June 16, 2005. Beetles were released in North Carolina (82,292), South Carolina (58,714), and Georgia (70,222). There were a total of 110 release sites across the tristate region. Starting in mid-May 2005, experimental releases of *S. tsugae* eggs were made and hatch success was monitored in the field. A total of 57,293 eggs on gauze were placed on HWA-infested hemlocks, with a hatch rate of 94.1 percent. Information was gathered on efficient number of eggs per clip cage, clip cage placement in the field, length of time clip cages should remain in the field, and last freeze date determination for egg releases to occur. Production of *S. tsugae* for 2006 began on December 27, 2005, with the goal of releasing a minimum of 100,000 adult beetles. In 2006, releases included 15,770 eggs and 167,189+ adults in North Carolina (4,136), South Carolina (30,050), and Georgia (133,003). We provided Young Harris College, in Georgia, with a start-up colony of 481 beetles as well as 350 beetles to Virginia Tech for imidacloprid studies. We have 9,387 adults in summer storage for the 2007 rearing season.

Clemson University began rearing *Laricobius nigrinus* in 2004-2005. A start-up colony of 200 beetles was provided in fall 2004 by the Salom lab at Virginia Tech and was supplemented in March 2005 with 500 *L. nigrinus* collected around Seattle, WA. The field-collected beetles produced 18,007 larvae. Adult emergence began in late August 2005 and a total of 3,000 beetles emerged. Beetles were released in Jackson (582) and Macon (277) Counties in North Carolina and in Rabun (835), Towns (182), Union (60), and White (245) Counties in Georgia for a total of 37 releases. We retained 700 beetles for colony use and obtained an additional 500 field-collected beetles from the Seattle area in March 2006. We produced 41,333 larvae, and as of July 2006 we have 38,586 adults aestivating in the soil. We are evaluating rearing techniques and preparing for releases in fall 2006.

Currently, we have a citizenship release-site monitoring program with the Jackson-Macon Conservation Alliance in North Carolina monitoring *S. tsugae* release trees. Other current research projects include investigating *S. tsugae* fitness when fed HWA from Carolina versus eastern hemlock, comparing HWA infestations on eastern versus Carolina hemlock, and investigating the plant chemistry of *Tsuga* spp. in relation to HWA feeding.

Cora Allard (864) 656-3136
Clemson University
callara@clemson.edu

Work on third Scymnus beetle progressing

Scymnus camptodromus is one of the three *Scymnus* predators of hemlock woolly adelgid imported from China that have been or are being developed for use as biological control agents. This species has an egg diapause that has limited our ability to rear and evaluate it previously. Recent research has shown that the diapause can be broken easily. Chilling the eggs at 5 °C for about 2 months will allow > 80 percent of the eggs to hatch at 10 °C. Methods are now being developed to rear this species so that host range testing and its efficacy against the adelgid can be evaluated.

Melody Keena (203) 230- 4308
USDA Forest Service
mkeena@fs.fed.us

CHEMICAL CONTROL

Aerial micronutrient application for hemlock health remediation

We tested the effects of an aerial foliar micronutrient application on hemlock foliar chemistry and vigor in mature stands of hemlock infested by HWA. Three sites in southern Pennsylvania were selected. At each site, two sets of paired plots (treated and control) were located in hemlock stands in a moderate state of decline and with light HWA infestation levels. A suite of physical, chemical, and physiological variables was measured in early spring (April) before the application of micronutrients. In addition, plots were sampled immediately after treatment (May) and at the end of the growing season (October). A short-lived treatment effect was observed for foliar Mg and starch immediately after treatment (May). However, this did not result in an increase in chlorophyll a or b, indicating that Mg was not a limiting factor for chlorophyll production. The short-lived increase in starch on treatment plots could be the result of increased photosynthetic activity that was not used for increased new growth. Initially following the micronutrient application, treatment plots experienced a significant decrease in overall decline symptoms that was not witnessed in the control plots. However, the micronutrient treatment appeared to have no long-term, positive effects on growth or vigor of infested hemlock trees in this study. Further, there is evidence for longer-term reduced growth rates and higher starch reserves on treated plots.

Richard Hallett (603) 868-7657
USDA Forest Service
rhallett@fs.fed.us

Research underway to assess nontarget impacts of imidacloprid

A 2-year research study was recently initiated to assess the nontarget impacts of imidacloprid (commonly used to reduce populations of HWA) on arthropod populations on eastern hemlock. A 2x5 factorial design with two application times (fall and spring) and five treatments (imidacloprid applied via stem/trunk injection, imidacloprid applied via soil injection, imidacloprid applied as a soil drench, horticultural oil, and an untreated check) was implemented on the Cherokee National Forest. Each treatment was applied in fall 2005 and in spring 2006 to better define seasonal nontarget impacts. Arthropod densities will be monitored regularly using beat sheets, direct observations and collections, sticky traps, malaise traps, surface litter collections, and pitfall traps. The imidacloprid content in foliage/needles will also be analyzed.

Jerome Grant (865) 974-0218
University of Tennessee
jgrant@utk.edu

Efficacy of the systemic insecticide imidacloprid applied with the Arborjet tree injection system to control HWA on eastern hemlock

The objective of this study was to evaluate the efficacy of the Ima-jet formulation of imidacloprid (5% a.i.) injected through the bark of the tree with the Arborjet Tree I.V. injection system to reduce populations of hemlock woolly adelgid. Trees used in this study were part of a native stand of eastern hemlocks in the Green Ridge State Forest in Allegheny County, Maryland. Eighteen eastern hemlocks were assigned to one of two treatments. Half received trunk injections of imidacloprid and the other half served as untreated controls. Trees were treated on May 27, 2005. The DBH of each tree was measured and imidacloprid was applied using the label recommended rates of 2 ml for trees less than 11 inches DBH and 4 ml for trees between 12 and 23 inches DBH. Diameters of all trees ranged from 8-20 inches at DBH. At the time of application, two branches infested with adelgids were marked with plastic tags on each tree. On the distal 30 cm of each branch, the number of adelgids was counted. These counts were combined to give an adelgid density expressed in number of adelgids per 60 cm of branch. Counts were repeated on October 20, 2005, and June 13, 2006, on each tree.

The results of the analysis of pretreatment count data indicate that HWA densities did not differ between treated and untreated hemlocks ($F_{1,16} = 0.24$, $P < 0.63$). The non-parametric analysis of counts taken 5 months after the application of imidacloprid detected marginally significant differences in the densities of HWA on treated or untreated trees ($\chi^2 = 3.17$, $P< 0.07$). Counts

taken in June 2006 revealed a significant reduction in the number of adelgids on trees injected with imidacloprid ($F_{1,15} = 8.16$, $P < 0.01$). There is evidence that injecting imidacloprid using the Arborjet Tree I.V. system reduces the abundance of hemlock woolly adelgid within one year of treatment by about 60 percent. Trees will be sampled again in the fall of 2006 to determine if this trend continues.

Michael Raupp (301) 405-8478
University of Maryland
mraupp@umd.edu

Imidacloprid dose response and tree size

Rich Cowles has discovered through a dose-response field trial in Pennsylvania that optimal dosing of hemlock trees with imidacloprid to control hemlock woolly adelgid is related to the size of the tree in a manner not apparent from the Merit label directions. The log of the optimal dosage is directly proportional to the diameter of the tree, whereas label directions give a range of dosages that are proportional to the tree's diameter. Therefore, small hemlock trees could be effectively treated with lower than labeled quantities of insecticide, whereas very large trees may require two or more successive treatments to achieve an effective titer of insecticides in their tissues. He has also discovered that soil-applied imidacloprid for controlling hemlock woolly adelgids has unexpectedly long-term benefits for managing this pest. Trees treated in the fall of 1999 are still showing a 90 percent reduction in adelgid populations relative to the untreated check trees.

Best Management Practices guidelines being developed for treating hemlock woolly adelgids now take long-term control into account: the suggestion is to not re-treat hemlocks (unless they are very large trees) until at least 2 years following an initial soil application, and only when adelgids have reappeared.

Rich Cowles (860) 683-4983
Connecticut Agricultural Experiment Station
richard.cowles@po.state.ct.us

Lethal and sublethal effects of imidacloprid on *Laricobius nigrinus* and *Sasajiscymnus tsugae*, two predators of HWA

Systemic injections of imidacloprid are widely used to control HWA. Two predators, *Laricobius nigrinus* (Coleoptera: Derodontidae) and *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae), are being mass reared and released as biological control agents. In laboratory bioassays, predator adults fed on HWA that survived on hemlock branches treated with imidacloprid. Both beetle species exhibited sublethal effects such as twitching, spasms, and paralysis. Both beetle species also exhibited a dose-dependent relationship between concentration and mortality. Temperature was a significant factor, with greater predator mortality at higher temperatures. At 4ºC, *L.*

nigrinus mortality ranged from 4 to 45 percent, and at 12º it was from 45 to 76 percent. *Sasajiscymnus tsugae* mortality ranged from 33 to 48 percent and 25 to 100 percent at 12 and 20º C, respectively. There was no control mortality for *L. nigrinus*; however *S. tsugae* had 25 percent mortality at 20ºC. Mortality increased over time at each concentration with more rapid rates at the warmer temperatures.

B.M. Eisenback (540) 231-8945
Virginia Tech
beisenba@vt.edu

SURVEY AND MONITORING

Standardized sampling for detection and monitoring of HWA within hemlock stands

Foresters and researchers have lacked a standardized sampling plan for HWA. The hemlock woolly adelgid sampling plan (HSP) is used to detect HWA in hemlock stands and determine the percentage of infested trees. By rapid examination of branches on 8 to 100 trees, HWA infestations can be characterized and as few as 2 percent infested trees can be detected with 75 percent reliability. The cutoff thresholds to stop sampling are based on optimum samples sizes to obtain a relative precision level of 0.25. Additional data are being collected and analyzed to further characterize the plan's functionality.

The HSP was specifically designed as a standardized protocol intended for widespread adoption in HWA surveillance activities. The first workshop for training forestry personnel in its use was conducted during 2004 in Massachusetts. Attendees from Massachusetts, Maine, New Hampshire, New York, and Vermont rated the plan's usefulness highly in the post-training survey. During 2004, the HSP was used on at least 97 sites in New York and the USDA Forest Service examined 92 stands in the Allegheny National Forest and 50 stands in the Monongahela National Forest. The Allegheny stands took a three-member crew 2 weeks to complete. During 2005, the HSP was used more widely and three additional workshops were conducted, along with presentations to regional forestry personnel.

The sampling plan training was also incorporated into a "Take A Stand" program spearheaded by Don Ouellette of the Maine Forest Service. After an initial HSP training and field exercise, citizen volunteers undertake HWA detection and monitoring on designated sites. The role of HSP beyond detection and monitoring will depend on its adaptation to decisionmaking processes related to HWA management, silvicultural practices, predator and pathogen releases, implementing salvage cuts, and ground truthing hyperspectral analysis for HWA detection. The adoption of HSP as a standard for surveillance should benefit monitoring HWA range expansion and research on its spread throughout Eastern North America.

Scott Costa (802) 656-2824
University of Vermont
scosta@uvm.edu

HEMLOCK RESISTANCE

Field trial of hemlock species and hybrids for resistance to HWA

Resistance of hemlock species and their hybrid crosses to HWA is being evaluated by the U.S. National Arboretum at the South Farm of the USDA, ARS Beltsville Research Center. This trial evaluates hemlocks for desirable growth characteristics and determines resistance to HWA. The hemlocks were produced from seeds obtained from controlled crosses, and then transplanted into the field nursery 4 years ago. The trees are currently 1 to 3 meters tall. The trial includes *Tsuga canadensis*, *T. caroliniana*, *T. chinensis*, *T. sieboldii*, and hybrids between both *T. caroliniana* and *T. chinensis* and *T. sieboldii* and *T. chinensis*. At least 10 replications of each species or hybrid combination were planted in the nursery. Hybrid crosses of Asian species with *T. canadensis* were not successful, so these combinations were not available. Survival and growth of *T. caroliniana* in the nursery has been poor. *T. chinensis* and the hybrids *T. chinensis x caroliniana* and *T. chinensis x sieboldii* have good survival and vigorous growth. The hemlocks were inoculated with crawlers of the progrediens generation this spring, and establishment and development are being monitored. Another aspect of the project is clonal propagation of the most promising hybrids for further evaluation in forest and ornamental plantings.

Susan Bentz (301) 344-4113
National Arboretum
sbentz@ars-grin.gov

Ex situ gene conservation of Carolina and eastern hemlock

Beginning in 2003, Camcore (an international tree conservation and domestication program at North Carolina State University) entered into an agreement with the USDA Forest Service to conduct a three-phase project on the *ex situ* conservation of hemlocks in the Eastern U.S. Phase 1 involves Carolina hemlock seed collections in the mountains and upper Piedmont regions of the Southeastern U.S. and is ongoing. Seed from 67 open-pollinated families was collected from 10 Carolina hemlock provenances in 2003 and 2005. The seed has been processed and catalogued for long-term storage. Representative samples have been sent to cooperators in Chile and Brazil who are growing Carolina hemlock seedlings in nursery facilities for eventual out-planting into *ex situ* conservation banks. Similar conservation banks will be established by the USDA Forest Service in the Ozark Mountains of Missouri and Arkansas. In 2006, Camcore plans to add seed from five additional Carolina hemlock provenances to its hemlock seed bank.

Phase 2 started in 2005 and is a 4-year project to collect seed from eastern hemlock populations in the Southeastern U.S. Currently, Camcore is selecting sites and assessing the 2006 cone crops. Seed from 20 eastern hemlock provenances will be collected in 2006 with additional

populations to be added during years 2 through 4. Similar to Phase 1, eastern hemlock seed with be processed and catalogued for long-term storage and the establishment of *ex situ* conservation banks in Chile, Brazil, and the Ozark Mountains. Phase 3 of the project will sample eastern hemlock populations in the Northeastern and Upper Midwestern regions of the U.S. and is tentatively scheduled to begin in 2009.

Robert Jetton (919) 515-6425
North Carolina State University
rmjetton@ncsu.edu

Genetic diversity studies of Carolina and eastern hemlock

In addition to *ex situ* conservation seed collections, Camcore is studying the genetic diversity of Carolina and eastern hemlocks. We are using AFLP molecular markers to assess the genetic structure of Carolina hemlock throughout the Southeastern U.S. Additionally, foliar and winter bud samples from eastern hemlock were collected from across the Southeast during February 2006, and chemical and molecular markers will be used to quantify the genetic diversity present in interior versus peripheral populations of the species. This study is in collaboration with Valerie Hipkins (Director of the National Forest Genetics Laboratory in Placerville, CA), Barbara Crane (National Forest System Regional Geneticist for Region 8), and Rusty Rhea (FHP Entomologist in Region 8). A second study of eastern hemlock is also being initiated in collaboration with Dr. John Frampton (North Carolina State Christmas Tree Genetics Program) and Dr. Dana Nelson (USDA Forest Service) to develop microsatellite markers for the species. Understanding the genetic diversity of hemlocks will guide us in establishing effective collection protocols for our *ex situ* conservation program.

Robert Jetton (919) 515-6425
North Carolina State University
rmjetton@ncsu.edu

IMPACTS

Delaware Water Gap National Recreation Area crown health monitoring

In 1993-1994, a total of 81 permanent plots were established in seven areas of the Delaware Water Gap National Recreation Area in Pennsylvania and New Jersey (established and monitored by Rich Evans, National Park Service, and Brad Onken, USDA Forest Service). Annual ratings of crown health were made on 10 hemlocks in each plot using the Forest Service Forest Health "Visual Crown Rating" guide. In 1998, decline in hemlock health was extensive in some areas but in other areas most hemlocks were still healthy. By 2001, all areas had noticeable decline. By 2005, 26 percent of the hemlocks surveyed were dead and those surviving had much thinner crowns (44 percent transparency compared to 22 percent in 1994) and much

more branch dieback (27 percent versus 4 percent in 1994). Although the decline in hemlock health has been slow and the rate of decline varies with area, currently no areas have any hemlock rated as fully healthy.

Mike Montgomery (203) 230-4331
USDA Forest Service
memontgomery@fs.fed.us

Stand dynamics associated with long-term HWA infestations

Vegetation dynamics have now been monitored for 10 years in eight hemlock stands with varying levels of HWA damage in south-central Connecticut to examine overstory hemlock mortality and subsequent community reorganization patterns associated with chronic HWA infestations. In 1995, overstory and understory mortality in seven infested stands ranged from 15 to 97 percent, and only two stands had > 80 percent mortality. In 2005, all eight stands were infested and seven had > 80 percent overstory mortality. There has been no sign of tree recovery at these stands and the health and vigor of remaining trees deteriorated in all infested stands. Results suggest that trees on some sites are still alive 12 years following initial infestation but many of the dead hemlock are no longer standing. Four additional stands in central and northern Connecticut have also deteriorated since 1998, but currently have lost only 15 to 35 percent of the overstory hemlock stems. The primary replacement species continues to be black birch, and some individuals are now 4 to 8 m tall.

David Orwig (978) 724-3302 ext. 250
Harvard Forest
orwig@fas.harvard.edu

Comparison of HWA vs. logging impacts

Many landowners choose to pre-emptively harvest their hemlock stands with the threat of HWA. Information from timber harvesters, State agencies, and Harvard Forest studies indicate that the recent broad-scale increase in logging associated with HWA is occurring with little ecological assessment and in the absence of scientific background for conservationists, land managers, or policy makers. We recently compared the impacts of hemlock harvest with those of HWA on the magnitude and trajectory of community and ecosystem dynamics. Plots were established at 10 sites throughout central Massachusetts on public and private lands where hemlocks had been harvested within the last 15 years at varying intensities. Recently cut forests exhibited higher soil temperatures, total nitrogen availability, and nitrate availability than forests cut 8 to 13 years ago. Similar to work conducted in Connecticut, hemlock forests that have been logged in Massachusetts are being replaced by black birch, a few oak species, and raspberry.

Logging and chipping treatments also occurred in the winter of 2005 on Hemlock Hill at Harvard University's Arnold Arboretum. Logs were removed by crane from four of six plots, ground into chips, and then spread onto two of the logged plots to see their impact on vegetation dynamics in this urban park environment. We measured nitrogen dynamics, microenvironment conditions, and vegetation dynamics this summer as part of the second year post-cutting. Hemlock Hill soils exhibited up to 20 times the available nitrogen and significantly lower soil pH values when compared to our other hemlock study sites in Connecticut and Massachusetts, illustrating the impact of higher nitrogen deposition at this urban site. Additional adelgid-resistant Chinese hemlock (*Tsuga chinensis*) has been planted on Hemlock Hill as part of a long-term revegetation effort. To provide a rural example of hemlock cutting and to simulate HWA damage, logging and girdling treatments were also begun at the Simes Tract at Harvard Forest in Petersham, MA, where ecosystem effects of hemlock removal are also being investigated.

David Orwig (978) 724-3302 ext. 250
Harvard Forest
orwig@fas.harvard.edu

HWA impacts on ecosystem function in Southern New England

In 1998, we began examining the magnitude and duration of nitrogen (N) cycling dynamics in response to the stress and mortality caused by HWA at eight study sites in Southern New England. Differences in nitrogen availability between heavily infested and lightly infested sites were obvious for the first 4 years. Those differences recently declined, probably due to HWA declines resulting from cold winter temperatures in 2003 and 2004. Thinning canopies from heavy HWA damage resulted in increased light, soil temperature, and mineral soil moisture, and decreased forest floor moisture content. Heavily infested sites continue to have larger extractable NH_4-N and NO_3 – N pools, and significantly higher net nitrification rates than healthy hemlock forests. In addition, resin bags captured more NH_4-N and NO_3 – N in infested versus uninfested stands. Foliar decomposition rates in infested vs. uninfested forests were actually lower, due to the reduced organic soil moisture in deteriorating forests. In addition, decomposing foliage from infested forests had significantly higher N concentrations and lower C:N ratios, suggesting that HWA feeding resulted in alterations of litter chemistry as decomposition progressed.

David Orwig (978)-724-3302 ext. 250
Harvard Forest
orwig@fas.harvard.edu

The impact of HWA on throughfall chemistry and microorganism abundance

We followed up our 2002 study with an additional study at Mt. Tom, MA, examining the effects of HWA-affected and HWA-unaffected throughfall on litter type, leachate, and litter chemistry. Early in the season, adelgid infestation caused higher dissolved organic carbon (+24.6 percent), dissolved organic nitrogen (+28.5 percent), and potassium (+39.3 percent) fluxes and lower inorganic nitrogen fluxes (-39.8 percent) in throughfall and in adjacent litter solutions collected beneath infested trees compared to uninfested trees. Needle litter collected beneath uninfested hemlock had significantly lower N concentrations compared to needles collected beneath infested trees, while no difference in N concentrations was found in birch litter. Bacteria were significantly more abundant on hemlock and birch litter beneath infested trees, while yeasts and filamentous fungi showed no consistent response to HWA throughfall. Litter microcosms showed that less dissolved organic carbon was leaching from birch than from hemlock needles when exposed to HWA throughfall. Overall, NH_4-N and dissolved organic nitrogen leachate concentrations were higher from birch than from hemlock litter. Thus, HWA affected throughfall leading to qualitative and quantitative differences in nitrogen export from the litter layer. The N concentration of hemlock litter did not change with time but the N concentration in birch litter increased significantly during the course of the experiment, especially when HWA-affected throughfall was applied.

David Orwig (978)-724-3302 ext. 250
Harvard Forest
orwig@fas.harvard.edu

Range expansion and community impact of the HWA and EHS in Southern New England

We assessed the spread and community-level impact of the hemlock woolly adelgid (HWA) and elongate hemlock scale (EHS) on Southern New England hemlock forests by resurveying 141 hemlock stands across a 7,500 km^2 latitudinal transect running from coastal Connecticut to northern Massachusetts. All of these stands had been previously identified via aerial photography and surveyed in either 1997-98 (Connecticut) or 2002-04 (Massachusetts) for HWA and EHS density as well as hemlock stand vigor. We rated two branches on each of 50 trees per stand on a scale of 0-3 (0=none; 1=1-10 organisms/m branch; 2=11-100/m branch; 3=100+/m branch) for HWA and EHS density. We also assessed percent overstory hemlock mortality as well as percent canopy loss in each stand.

Canopy loss and hemlock overstory mortality increased with increasing HWA density, with the most damaged stands located in southern Connecticut and undamaged stands in northern Massachusetts. HWA density decreased with increasing latitude, possibly due to climatic limitations, and was absent from much of northern Massachusetts. Although the total number of HWA-infested sites increased between the initial and 2005 surveys, mean HWA density per site has decreased over this same period.

EHS distribution and density per site has increased dramatically since 1997-98 and is now present in every Connecticut stand and many Massachusetts stands. Within the survey transect, EHS appears to be moving rapidly northeast from the site of its initial introduction on Long Island in 1908. Despite its rapid increase in abundance, EHS density was not correlated with overstory hemlock mortality when the effect of HWA presence was taken into account.

There was a positive correlation between HWA and EHS density on trees within individual sites in sites with low overall HWA densities. As overall HWA density increased, however, this correlation shifted from positive to negative (likely as a result of resource competition). We are currently conducting experimental work to determine the nature of the relationship between these two herbivores.

Evan Preisser (413) 577-2478
University of Massachusetts
preisser@psis.umass.edu

Influence of eastern hemlock on stream macroinvertebrate communities in southwestern Virginia

Slow-growing, shade-tolerant eastern hemlock, *Tsuga canadensis*, is an important tree species associated with numerous eastern forest streams. The full impact of HWA on hemlock-associated stream ecosystems is unknown. We are examining the role of hemlock in southern Appalachian streams by studying macroinvertebrate community composition and hemlock litter decomposition. In spring, summer, and fall of 2005, benthic macroinvertebrates were collected from six stream sites with varying densities of riparian hemlock cover in Giles County, Virginia. In addition, hemlock needles (litter) were placed in each stream and collected over time to evaluate litter decomposition and macroinvertebrate attraction to hemlock needles. Initial results indicate that the macroinvertebrate community composition appears to be related to hemlock tree density. Low-density hemlock streams averaged fewer shredder macroinvertebrates (10 percent total organisms) compared to 50 percent shredders at a high-density hemlock site. Macroinvertebrates in the scraper functional feeding group conversely were more abundant in low-density hemlock streams. Hemlock litter had a slow decomposition rate (k=0.006) at all stream sites, making it available to macroinvertebrates for a longer period of time.

Meredith Worthen (540) 951-2536
Virginia Tech
mworthen@vt.edu

HWA BIOLOGY

DNA used to trace origin of the hemlock woolly adelgid in Eastern North America

We performed a molecular phylogenetic study using mitochondrial DNA to examine the relationship among adelgids found on hemlock worldwide (Havill et al. 2006 – pdf at http://pantheon.yale.edu/~nph3/). We were interested in determining the amount of genetic differentiation among hemlock adelgids found in different geographic regions and on different host hemlock species in order to locate the source of the introduced *Adelges tsugae* in Eastern North America. We found that there are several distinct evolutionary lineages of adelgids on hemlock worldwide. In Japan, there appears to be two different lineages associated with each of the two Japanese hemlock species. The adelgids in Eastern North America were clearly introduced from the population that is living on *Tsuga sieboldii* in lower elevations in southern Honshu, Japan. Adelgids from mainland China and Taiwan are quite diverged from those living in Japan and North America and should probably be considered a separate species. Finally, hemlock woolly adelgids living in Western North America appear to be a separate lineage that is endemic to that area. This has been further corroborated by a molecular phylogenetic analysis of the entire family Adelgidae that includes additional genetic markers (Havill, von Dohlen, and Foottit, in preparation) in which hemlock adelgids from Western North America form a separate, well-supported, monophyletic group with genetic variation similar to the two host-associated lineages in Japan.

Nathan Havill (203) 432-7394
Yale University
nathan.havill@yale.edu

Characterization of bacterial endosymbiont diversity in HWA

In cooperation with Carol von Dohlen at Utah State University, we have discovered and localized three Gamma-Proteobacteria endosymbionts in HWA. In Western North America, the bacteriomes of HWA seem to be occupied solely by Gamma-C. In Eastern North America, Gamma-C seems to occupy the majority of cells and cell area, taking up the outermost regions of the bacteriome and surrounding several central cells or cell regions that contain a second Gamma-Proteobacteria, the Gamma-B endosymbiont. An exciting finding was the discovery of the Gamma-B endosymbiont in a Japanese population on *Tsuga sieboldii*. The bacteriomes of this Japanese population show the same form as those of Eastern North America, with bacteriocytes containing Gamma-C surrounding central cells or cell regions containing Gamma-B. However, experiments with a nearby Japanese population (also on *Tsuga sieboldii*) show no evidence of Gamma-B bacteria. Specific PCR experiments to detect Gamma-B in this and two other Japanese populations (both from *Tsuga diversifolia*) have failed to detect this bacterium. Independent work by Nathan Havill on the phylogeography of HWA using adelgid host genes has determined that HWA in North America originated from Osaka populations on *T. sieboldii*. Thus, it seems that HWA in Eastern North America shares a unique endosymbiont (Gamma-B) with only one population in Japan. This is intriguing because it may pinpoint the origin of HWA

infestation to this one Japanese population. However, we need to survey more Japanese populations to be more certain of the putative localized occurrence of Gamma-B in Japan. The additional intriguing possibility arises that the presence of Gamma-B in HWA may be somehow correlated with the successful introduction and establishment (and/or virulence) of HWA in Eastern North America.

Kathleen Shields (203) 230-4320
USDA Forest Service
kshields@fs.fed.us

Biogeographic assessment of HWA and biological controls in the Eastern USA

In 2006, a new bioclimatic analysis of HWA was initiated. This study relates HWA survival to 1) landscape climatic and structural variation using climate data from the National Oceanic and Atmospheric Administration (NOAA) National Climate Data Center (NCDC), 2) SRTM90 DEMs, and 3) field-sampled HWA populations and local weather. Using the available NOAA NCDC climate data, the current range of HWA has been divided into 10 equally spaced bands within a thermocline. Two hemlock stands within each band will be sampled at multiple times during the winter of 2006-2007 to monitor adelgid development and mortality. Local conditions at each stand will be monitored using a micro station. These data should provide an improved estimate of the potential maximal range of HWA in the Eastern United States. Current estimates are based on the use of interpolated NOAA NCDC climate data using the current HWA distribution as a climate template. This preliminary approach indicates HWA has invaded roughly half of its total minimum potential range, though new data should improve this estimate. A similar approach, based on the use of published information on the temperature requirements of HWA biological control agents, is underway for *Sasajiscymnus tsugae, Scymnus camptodromus, Scymnus ningshanensis, Scymnus sinuanodulus, and Laricobius nigrinus* to estimate landscape suitability, based on landscape estimates of climatic conditions.

Talbot Trotter III (203) 230-4312
Northern Research Station
rttrotter@fs.fed.us

Effect of climatic variables on the ultimate distribution of HWA

Using 70 locations across the Eastern U.S., we have taken advantage of both latitudinal and continental gradients to show that ~60 percent of the variation in rates of winter adelgid mortality is explained by mean (30-year) minimum January temperatures based on a Krieged temperature estimate for the landscape using data from 2,800 weather stations. These results also suggest that the use of contemporary weather data from the weather station nearest to the point where adelgids were collected increases the explanatory power to ~90 percent. Temperature variation also appears to play a role in the survivorship of HWA, such that insect mortality

appears positively correlated with the difference in mean temperature extremes during winter months. These data suggest that the ultimate limits of adelgid range expansion can be estimated using landscape climatic analyses in combination with field observations, and that additional regional sampling can be used to refine the estimates of the potential maximal range of HWA in Eastern North America.

Kathleen Shields (203) 230-4320
USDA Forest Service
kshields@fs.fed.us

Life cycle of hemlock woolly adelgid in eastern Tennessee

A 2-year study was completed to better define the biology and seasonality of the hemlock woolly adelgid in the eastern Tennessee region of the Great Smoky Mountains National Park. In general, the development of this invasive pest was about 2 to 4 weeks earlier than that reported in similar studies in the Northeastern United States. Eggs were found from February to June, with densities peaking in late February/early March (sistens) and late May/mid June (progrediens). Crawlers, the most mobile stage, were present from late March to late June; densities of crawlers were highest from late March to mid April and again in late June. A better understanding of the life cycle of hemlock woolly adelgid in the Southeastern United States will enable scientists and land managers to enhance IPM programs. A Powerpoint copy of the life cycle is available upon request.

Jerome Grant (865) 974-0218
University of Tennessee
jgrant@utk.edu

Inverse density-dependence in hemlock woolly adelgid (*Adelges tsugae*) dispersal at low population density

Dispersal of the HWA in the Eastern United States is probably limited to eggs and crawlers. Tom Baribault (University of Vermont Ruby HELiX/EPSCoR Ruby intern), Phil LaBranche (Harvard Forest Summer Research in Ecology Program intern), and I examined crawler dispersal from hemlock trees in relation to adelgid density and tree health. In 2004, hemlock branches (16 of 1 meter) ranging in HWA infestation were selected and evaluated for growth, vigor, and HWA abundance and captured on ground-level sticky cards. In 2005, infested trees were randomly selected and HWA quantified, and 20 trees within a 30-yard radius of each tree were also surveyed for the presence of HWA.

Adelgid density and tree health appear to influence dispersal of HWA crawlers. The proportion of crawlers leaving a tree and spreading to adjacent trees is greater when HWA populations are lower (inverse density-dependence). Crawler capture on the sticky traps was also significantly

($P \leq 0.05$) correlated with tree health (Pearson's $r = 0.49$) measured as new shoot establishment. More crawlers leaving when trees are still healthy could be a response to hemlock defensive chemistry; hemlock health quickly deteriorates under adelgid attack. Inverse density-dependence in crawler dispersal could promote range expansion when HWA populations are initially establishing or experiencing stress.

Scott Costa (802) 656-2824
University of Vermont
scosta@uvm.edu

Understanding factors that regulate HWA population growth and spread

Our main goals are to 1) determine the most important factors regulating HWA population growth, 2) estimate the potential northern limits of HWA, and 3) determine the impact of *Sasajiscymnus tsugae* on HWA populations in Southern New England. To address these issues, we have been collecting data for the past 3 years on overwintering mortality, fecundity, density, tree health, hemlock elongate scale density, and winter temperature. These measurements were taken at six sites in Massachusetts and Connecticut—three *S. tsugae* release sites and three non-*S. tsugae* release sites.

By incorporating factors such as adelgid density, scale density, and tree health in a multiple regression analysis, we hope to better understand HWA population dynamics and use this information to manage outbreak areas and predict future spread. In collaboration with Katharine Hayhoe, an atmospheric scientist at the University of Illinois-Urbana, we are estimating the future potential range of the adelgid based on overwintering mortality, fecundity results, and previous cold-tolerance studies.

HWA overwintering mortality, measured at an additional eight sites in Connecticut and Massachusetts, has decreased remarkably since the winter of 2003-04, the first year of this study. Mortality was significantly correlated with temperature in 2003-04. While the same trend was observed the following winter (2004-05), the relationship was not significant. Data are still being analyzed for the winter of 2005-06. So far, nothing in our data indicates a difference in adelgid population growth rate between *S. tsugae* and non-*S. tsugae* sites, although data for the third year are still being collected and analyzed. Currently, we are examining the effect of biological control agents at a smaller spatial scale using whole-tree enclosure studies with the predators *Laricobius nigrinus*, *Sasajiscymnus tsugae*, and *Scymnus sinuanodulus.*

Annie Paradis (413) 545-1032
University of Massachusetts
aparadis@psis.umass.edu

Silvicultural Management

A new silvicultural options brochure has been published

Dave Orwig (Harvard Forest) and David Kittredge (University of Massachusetts, Amherst) have incorporated findings from Harvard Forest studies of HWA and hemlock logging into a pamphlet for landowners that provides silvicultural options for managing hemlock forests. Options range from doing nothing to light selection cutting to high-intensity cutting, depending on the landowner objectives, overall hemlock health, and stand conditions. In addition, the pamphlet includes planting options and addresses HWA Best Management Practices. All options and costs should be considered carefully when planning appropriate management strategies to effectively meet desired goals and make an informed decision about hemlock and the threat of HWA. See Orwig and Kittredge 2005 in the publication section.

David Orwig (978) 724-3302 ext. 250
Harvard Forest
orwig@fas.harvard.edu

Activities in States and on Federal Lands

New Hampshire

New Hampshire continues to monitor for HWA using four major surveying systems. The first is a "Service Call Program" where we mail out 5,000 to 10,000 brochures per year to targeted regions asking landowners to check their hemlocks. This generates over 100 calls per year to our office from people concerned that they found HWA. Of those calls, we have ground checked 50 to 75 sites per year and found HWA at one to five locations. The second survey system is our Deer Yard Survey. The Forest Health staff surveys 30 hemlock deer yards per year in the southern three counties. The third HWA survey system is our "Ponds and Parks" Survey. The Forest Health staff uses maps to identify high-risk hemlock sites in the southeastern section of New Hampshire and a survey is completed in approximately 30 of these sites per year. In 2006, we found HWA at five of these locations. The fourth method of surveying HWA is nursery inspections performed by the New Hampshire Department of Agriculture per order of the New Hampshire exterior quarantine. All hemlock nursery stock shipped into New Hampshire requires a phytosanitary certificate and may be subject to inspection before it can be sold. The New Hampshire Division of Forests and Lands (NHDFL) continues to enforce an interior quarantine on the movement of hemlock forestry material out of Rockingham County. All hemlock logs and pulp must be inspected by a certified inspector before it may move to other regions of the State. Approximately 200 timber sales per year require this inspection.

New Hampshire has recently completed a Federal Environmental Assessment of the current treatment options available in the State. When this document is approved, the NHDFL will have chemical, biological, and mechanical options to implement at any of the HWA infestation sites. Currently, we have 12 different infestation sites and all will receive some type of treatment.

Kyle Lombard (603) 271-7858
New Hampshire Division of Forests and Lands
klombard@dred.state.nh.us

New Jersey

A late-winter survey of adelgid-infested sites indicates high mortality (~90 percent) of HWA at many sites. One exception is hemlocks along the Delaware River at Worthington State Park where healthy HWA populations were observed. Three sites—two in Warren County and one in Sussex County—have been chosen for *Scymnus* releases. A release of 500 *Scymnus* was made at Dunfield Creek on May 19.

On June 6, sticky board traps were placed at various levels within 33 infested hemlock trees along the Delaware River at Worthington State Park to survey for *Scymnus* and *Sasajiscymnus*. The traps will be retrieved in about 5 weeks.

Although most of our effort will be to produce as many *Scymnus sinuanodulus* as possible, the colony of *Sasajiscymnus tsugae* remains strong and vigorous. One *S. tsugae* release is planned for New Jersey this season.

Bob Chianese (609) 530-4194
New Jersey Department of Agriculture
robert.chianese@ag.state.nj.us

Maryland

The Maryland Department of Agriculture, Forest Pest Management Section conducted HWA suppression on areas prioritized by the HWA Task Force. To date, we have injected the trunks of 218 trees (3,600 dbh). Soil injections have been more productive, and we have treated 2,252 trees (23,000 dbh). In conjunction with the landowner incentive program, we used soil injection on 382 trees (4,000 dbh) on The Nature Conservancy property. All stands are evaluated for post-treatment efficacy.

To monitor HWA impacts, nine permanent plots are monitored each year. In an effort to detect new infestations or increasing HWA populations, Forest Pest Management staff scout priority stands, mainly in western Maryland.

On the biological control front, releases since 1999 have included *S. sinuanodulus* beetles at two sites, *S. tsugae* at 3 sites, and *L. nigrinus* released at four sites. No significant recoveries have been made, except for *L. nigrinus* at Rocky Gap State Park where it is found established and reproducing quite well. We hope to use this site as a field insectary.

Steve Tilley (800) 413-8828
Maryland Department of Agriculture
tilleysa@mda.state.md.us

West Virginia

In 2006, the West Virginia Department of Agriculture (WVDA) continued to monitor three permanent study plots in Blackwater Falls State Park, Cathedral State Park, and Greenland Gap. Detection surveys located HWA in Barbour, Braxton, Boone, and Kanawha Counties, bringing the total number of infested counties to 28. Due to limited availability of predators, only one release consisting of 450 *Scymnus sinuanodulus* beetles was made in Calvin Price State Park. The WVDA continued to monitor eight previous release sites of *Sasajiscymnus tsugae* and the single *S. sinuanodulus* release site. Beat sheet sampling of the two *Laricobius nigrinus* release sites is scheduled for this fall. Approximately 250 high-profile and high-value hemlocks on State lands were treated with imidacloprid via soil and stem injection this spring.

Karen Kish (304) 558-2212
West Virginia Department of Agriculture
E-mail: kkish@ag.state.wv.us

Great Smoky Mountains National Park

The Great Smoky Mountains National Park contains over 800 acres of old-growth eastern hemlock (*Tsuga canadensis*), more than any other National Park Service unit. Hemlock forests are widely distributed over almost 90,000 additional acres in the park. The Smokies also have some of the largest eastern hemlocks known, commonly exceeding 150 feet tall and as much as 6 feet in diameter. In 2002, HWA was identified in the park. This insect has now been identified throughout the park and has the potential to eliminate hemlock trees from the landscape. The Shenandoah National Park has lost almost 95 percent of its hemlocks due to HWA.

The park has aggressively managed HWA since its discovery. In 2006, the HWA crew consisted of 12 employees. Supported by funds from the Friends of the Smokies, USDA Forest Service, and National Park Service, park managers treated over 2,000 acres in 2006. Treatments included spraying insecticidal soap on trees in developed areas and along roadways, and applying

systemic insecticides directly into the soil or trunks of larger or inaccessible trees. Over 15,000 trees were treated with systemic insecticide on about 250 acres. The treatment results have been dramatic. Trees with ashen gray foliage prior to treatment produced new growth and recovered their color.

Additionally, park biologists released 65,849 predatory beetles (*Sasajiscymnus tsugae*) as a biological control in 28 old-growth areas throughout the park. There were 1,418 *Laricobius nigrinus* beetles released, including 800 to establish a field insectary. Although it is too early to determine the effect of the predators, this and other biocontrols currently under development provide the best hope for landscape-level control of the adelgid and survival of the hemlock forests.

Tom Remaley (865) 436-1252
Great Smoky Mountains National Park
tom_remaley@nps.gov

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USDA Forest Service
Northeastern Area State and Private Forestry
11 Campus Boulevard, Suite 200
Newtown Square, PA 19073

www.na.fs.fed.us